Docket No.: 223002010005
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Michael HOUGHTON et al.

Application No.: 09/884,456

Filed: June 18, 2001 Art Unit: 1656

For: HEPATITIS C VIRUS PROTEASE Examiner: W. Moore

DECLARATION OF J.-H. JAMES OU
UNDER 37 C.F.R. § 1.132

I, J.-H. James Ou, declare and affirm that:

1. I am currently Professor of Molecular Microbiology & Immunology in the Keck School of Medicine at the University of Southern California, Los Angeles, California.

2. I have a Ph.D. in Molecular Virology from California Institute of Technology, Pasadena. Since 1986 I have been involved in research on hepatitis viruses. A copy of my curriculum vitae and list of publications is attached.

3. I am not an inventor of U.S. Application No. 09/884,456.

4. I have read the above referenced U.S. Application No. 09/884,456, and I understand the subject matter contained therein. I am qualified to comment on what one of ordinary skill in the art would understand from reviewing the disclosure of this application and the publications referred to in my declaration.

A. U.S. Application No. 09/884,456 discloses a HCV protease

5. I have reviewed U.S. Application No. 09/884,456 ("the '456 application") and believe that one of skill in the art would understand that the specification discloses a Hepatitis C Virus (HCV) protease.

6. The specification states that: "[t]he term 'HCV protease' refers to an enzyme derived from HCV which exhibits proteolytic activity, specifically the polypeptide encoded in the NS3 domain of the HCV genome." (page 6, lines 22-24)

7. An HCV NS3 domain protease sequence is provided in Figure 1 of the application specification. (page 3, line 7).

8. An HCV protease encoded by the NS3 domain in at least one strain of HCV is further described with reference to a 202 amino acid protease sequence from SEQ ID NO: 1 in page 6, line 26 to page 7, line 18 (SEQ ID NO: 65).

B. U.S. Application No. 09/884,456 discloses a HCV NS3 domain serine protease

9. The specification describes an NS3 domain of HCV. Page 5, line 11 to page 6, line 4 refer to NS3 domain by analogy with the Yellow Fever Virus (a flavivirus) polyprotein. The specification points to a specific section in the NS3 domain as the key to proteolytic activity. (page 7, line 19 through page 8, line 6).

10. Pages 8-9 identify active residues responsible for protease activity by alignment with related serine protease sequences. Table 1 of the '456 application discloses HCV peptides from within SEQ ID NO: 65 and by alignment with catalytic residues of flavivirus serine proteases, identifies His-1083, Asp-1107 and Ser-1165 of the HCV genome as active residues for serine protease activity. Table 2 confirms the identification of the same residues by structural alignment with well-characterized serine proteases.

11. Examples 10 and 11 (pages 37- 39) provide methods for expression *in vitro* of HCV protease as a method for confirming the disclosure of HCV NS3 serine protease.

12. Eckart *et al.* (Biochem. Biophys. Res. Commun. 192:399-406 (1993).) used an *in vitro* transcription translation system to demonstrate a protease activity encoded by the NS3 domain that was mediated by the Ser-1165 residue identified in Table 1 of the specification.

(i) NS3 serine protease cleaves several substrates in the absence of the cofactor NS4A

13. It is now known that the NS3 serine protease cleaves the HCV polyprotein at multiple sites – NS3/4A, NS4A/4B, NS4B/5A and NS5A/5B. Only the NS4B/5A cleavage is dependent on the presence of NS4A. Bartenschlager *et al.* (J Virol. 68(8):5045-5055 (1994)). Bartenschlager has also shown that the first 211 amino acids of NS3 were sufficient for processing at all *trans* sites.

14. The NS3 serine protease mediated cleavages at NS3/4A, NS4A/4B and NS5A/5B are processed efficiently in *trans* by the NS3 serine protease without NS4A as follows:

> By using an NS3-5B substrate with an inactivated serine proteinase domain, trans-cleavage was observed at all sites except for the 3/4A site. Deletion of the inactive proteinase domain led to efficient trans-processing at the 3/4A site. Smaller NS4A-4B and NS5A-5B substrates were processed efficiently in trans; however, cleavage of an NS4B-5A substrate occurred only when the serine proteinase domain was coexpressed with NS4A.

Abstract, Lin *et al.*, J. Virol. 68(12): 8147-8157 (1994).

15. I have reviewed Sardana *et al.* (Protein Expression and Purification 16:440-447 (1999)). I understand Sardana to show that the NS4A cofactor is essential for "high" proteolytic activity of the NS3 serine protease. (*see* Abstract). Sardana acknowledges that proteolysis at the NS4A/4B junction is carried out at detectable levels by the NS3 serine protease in the absence of NS4A. (Sardana at 443, left col.).

16. Vishnuvardhan *et al.* (FEBS Lett. 402(2-3):209-212 (1997)) shows that a NS3 serine protease representing amino acids 1027–1218 of the HCV polyprotein, and not including any NS4A region, cleaves the NS5A/5B junction in the absence of NS4A. (Figs. 1 and 3). NS4A (amino acids 1658-1712; *see* Fig. 1) enhances the cleavage but is not essential for it. (Fig. 3).

Further, Vishnuvardhan classifies the NS4A/4B cleavage site as "NS4A-independent" cleavage site. (at 211).

17. Barbato *et al.* J. Mol. Biol. (1999) 289, 371-384, at 372, left col. states that "[i]nteraction with the NS4A cofactor is required to perform the cleavages at NS3/NS4A, NS4A/NS4B and NS4B/NS5A junctions but the proteinase in its uncomplexed state is still able to cleave at the NS5A/NS5B boundaries, although with a much lower activity."

18. The functionally minimal domain required for activity of the NS3 serine protease is composed of 146 amino acids, 1059 to 1204. (Yamada *et al.* Virology 246: 104-112 (1998)).

19. Figure 1 and SEQ ID NO.1 (page 6-7) of the '456 application discloses a sequence that encompasses the entire minimal domain of the NS3 serine protease.

20. The NS3 minimum domain can function as a protease from a structural point of view. Love *et al.* Cell 87: 331-342 (1996).

(ii) Polyprotein substrates for NS3 serine protease activity are disclosed in the Specification, and viral polyprotein substrates for proteases were commonly used in the art

21. Protease assays using *trans*-cleavage of viral polyprotein substrates were known in the art at the time of the filing of the invention. The following examples show the widespread use of viral polyproteins as substrates for viral proteases prior to 1990.

- Processing of a 250 kDa Sindbis Virus polyprotein substrate (S1234) *in vitro* by Sindbis Virus protease prepared by *in vitro* translation. de Groot, *et al.* The EMBO J. 9(8)2631-2638 (1990).
- *Trans* cleavage of a poliovirus capsomer precursor protein by poliovirus Proteinase 3C. Nicklin: J. Virol (1988) 62: 4586-4593.
- *Trans* assay of MLV protease using Gazdar murine sarcoma virus (Gz-MSV) polyprotein Pr65(gag) substrate. Yoshinaka, Proc Natl Acad Sci U S A. (1985) 82(6):1618-1622.

- *Trans* assay of FeLV protease using Gazdar murine sarcoma virus (Gz-MSV) polyprotein Pr65(gag) substrate. Yoshinaka, J. Virol. (1985) 55(3):870-873.
- *Trans* assay of BLV protease using Gz-MSV polyprotein Pr65(gag) and BLV gag precursor protein substrates. Yoshinaka *et al.*, J. Virol. (1986) 57(3):826-832.
- The proteinase of human immunodeficiency virus (HIV), expressed in Escherichia coli, shows rapid, efficient, and specific cleavage of an *in vitro* synthesized gag precursor polyprotein. Kräusslich *et al.,* Proc Natl Acad Sci U S A. (1989) 86(3): 807–811.
- Processing of HIV-1 Pr53(gag) polyprotein substrate in *trans* (Fig. 4) by HIV2 protease fusion proteins from bacteria and yeast. Pichuantes *et al.* J. Biol. Chem 265(23):13890-13898 (1990).
- A fusion protein comprising HIV1 protease fused with human superoxide dismutase (hSOD) expressed in yeast displayed correct self-processing, and *trans*-processing *in vitro* of a gag-precursor $Pr53^{gag}$ polyprotein substrate. (*see* Fig. 4, Table 1, Pichuantes *et al.*, Proteins. 6:324-37 (1989))

22. A substrate for the serine protease activity in the form of genomic HCV polyprotein is disclosed in page 20, lines 14-16 of the specification. Page 21, lines 4-5 explains that "[i]n the absence of this protease activity, the HCV polyprotein should remain in its unprocessed form."

23. A method for inactivating the HCV protease activity by a single point mutation "substituting Ala for Ser_{121}" is disclosed in page 22, line 27 to page 23, line 15 of the specification. One of skill in the art would have understood that this method can be used to inactivate the NS3 serine protease activity of the genomic HCV polyprotein – such that it can then be used as a substrate for testing NS3 serine protease activity in *trans*.

24. Lin et al. used such a substrate with an inactivated serine proteinase domain to assay trans-cleavage by NS3 serine protease. (Lin *et al.*, J. Virol. 68(12): 8147-8157 (1994))

25. One of skill in the art would understand that the '456 application describes a NS3 serine protease based on comparison with related flavivirus proteases and identification of critical amino acid residues of the serine triad. One of skill in the art would also understand that a substrate for the NS3 serine protease activity in the absence of NS4A cofactor is disclosed in the '456 application in the form of genomic HCV polyprotein.

C. An NS2/3 protease activity associated with the HCV NS3 domain is disclosed in the Specification.

26. Example 5 of the Specification of the '456 application discloses specific NS2/3 cleavage by hSOD-NS3 domain fusion polypeptides.

- A protease activity is characterized in Example 5 (page 31, line 5 to page 32, line 12) which shows cleavage of hSOD–HCV protease fusion proteins expressed in *E. coli*. Page 31, lines 12-13 states that "[t]he results indicated the occurrence of cleavage, as no full length product (theoretical M_r = 93 kDa) was evident on the gel."
- Example 4 (page 29, line 4 to page 31, line 3) describes the amino acids of HCV protease encoded by each fusion protein.
- The P190 fusion product encoding amino acids 1-199 of the HCV protease (page 29, lines 19-20) showed no protease cleavage activity (page 32, lines 8-10).
- P300 which includes amino acids 1-299 of HCV protease (page 29, lines 25-26; SEQ ID NOS: 66 and 68) indicated occurrence of cleavage (page 32, lines 1-2).
- P500 comprising amino acids 1-513 of Fig. 1 (page 30, lines 4-6) indicated occurrence of cleavage (page 31, lines 22-25).
- The fusion protein ("P600") encoded by the vector cf1SODp600 which includes amino acids 1-686 of Fig. 1 (SEQ ID NO: 70) also showed proteolytic activity. (page 29, lines 5-14; and page 31, lines 12-17).

- The specification concludes that "the minimum essential sequence for HCV protease extends to the region between amino acids 199 and 299." (page 32, lines 10-12).

27. One of skill in the art would understand from reviewing the '456 application that a protease activity associated with a specific segment of HCV polyprotein is disclosed in the specification.

(i) The 34 kDa protein shown in Example 5 of the Specification is not inconsistent with self-cleavage product of the NS2/3 protease fusion protein

28. A 34 kDa band corresponding to a product comprising hSOD and part of the NS3 domain is consistently observed with fusion proteins P300, P500 and P600, but not with P190 which lacks the activity of NS2/3 protease.

29. Determination of exact molecular weight by SDS-polyacrylamide gel electrophoresis can be unreliable. It is now known that the specific NS2/3 self cleavage product comprises 151 amino acids of hSOD and amino acids 1-82 shown in Figure 1. The theoretical size of this fragment without any post translational processing would be expected to be about 25 kDa. However, in Example 5, the 34 kDA size is estimated from a Western blot of a SDS-polyacrylamide gel.

30. While SDS-polyacrylamide gel electrophoresis is often used to estimate molecular weights of proteins by comparing migration of proteins relative to a set of standard markers, it is well-known in 1991 that proteins and proteases do not necessarily migrate on SDS-polyacrylamide gels according to their predicted molecular weight.

- "[A]bnormalities in SDS binding or protein conformation, large differences in intrinsic protein charge, ... may lead to increased or decreased electrophoretic mobilities; therefore caution is advisable in use of this technique." Proteins: Structural and Molecular Principles. T. Creighton. page 33. (WH Freeman and Co., New York, © 1984).

- "[D]iscrepancy between apparent relative masses and real molecular weights underlies the uncertainty in deducing molecular masses of membrane-bound proteins from their mobility in electrophoretic gels." Introduction to Protein Structure. Brande C., and Tooze J. page 204 (Garland Publishing, Inc. New York and London © 1991).

31. Several proteases are known to migrate according to anomalous molecular weights in SDS-polyacrylamide gel electrophoresis:

- A NS2B-NS3 fusion protein from Dengue virus – a member of the flavivirus family which includes HCV – with a predicted molecular weight of 29.8 kDa displays anomalous migration in SDS-polyacrylamide gel electrophoresis with a higher apparent molecular mass of 37 kDa. Niyomrattanakit P., et al. J. Virol. (2004) 78(24): 13708-13716, at 13711, left column.

- A serine protease with a predicted molecular weight of 24.205 kDa was found to migrate at greater than 26 kDa possibly due to "the presence of bound [protein] defensin, possible posttranslational modifications of the protease, incomplete reduction of the protease during sample preparation or any combination of these possibilities." Hamilton JV et al., Insect Molecular Biology (2002) 11(3): 197–205, at 201, right column.

32. The specification shows that estimates of molecular weights of known proteins from SDS-polyacrylamide gel electrophoresis were not precisely according to the predicted theoretical size. For example, the molecular weight of the 151 amino acid hSOD partner by itself was estimated by gel electrophoresis to be about 20 kDa on page 31, lines 15-16, whereas its theoretical size is 16.5 kDa.

33. From the consistent observation of a 34 kDa band reactive to anti-HCV antisera described in Example 5 of the specification of the '456 application, corresponding to the active fusion proteins P300, P500 and P600, but not with the inactive P190 fusion, one of skill in the art

would have understood the "34 kDa" band is not inconsistent with a product of specific cleavage by the NS2/3 protease.

(ii) Stable and active viral protease fusion proteins were known in the art prior to 1991

34. It is known that fusion of heterologous sequences to the N-terminus of proteases does not affect the proteolytic activity of the protease. Human Immunodeficiency Virus (HIV) proteases remain active when a heterologous sequence is added to either terminus. The fused proteases mediate self-cleavage of viral polyproteins at the correct cleavage sites.

- A fusion protein comprising sequences from chloramphenicol acetyltransferase enzyme and HIV-1 protease is capable of autoprocessing, and mutation of the active site residue results in incorrect cleavage. Montgomery *et al.*, Biochem. Biophys. Res. Comm., 175(3):784-94 (1991).
- An HIV protease fused to the amino or carboxy terminus of bacterial ß-galactosidase retains its capacity for specific autoprocessing. Valverde *et al.*, J. Gen. Vir. 73:639-51 (1992)

35. As of the filing date of the parent application, April 4, 1991, fusion of a protein of interest to human superoxide dismutase (hSOD) sequence was an established method of achieving high-level expression of a stable fusion protein. The specification of the '456 application discloses expression of HIV protease as a fusion with human superoxide dismutase (hSOD) and having autocatalytic proteolysis activity by Pichuantes *et al.*

36. Prior to 1991, examples of HIV proteases fused with hSOD and showing proteolytic activity for self-cleavage as well as cleavage using viral polyprotein substrates in *trans*, had been observed:

- hSOD-HIV2 protease fusion from bacteria correctly self-processes and processes HIV-1 Pr53(gag) polyprotein in trans (Fig. 4). Pichuantes *et al.* J. Biol. Chem 265 (23):13890-13898 (1990)

- A fusion protein of HIV1 protease with human superoxide dismutase (hSOD) expressed in yeast displayed correct self-processing, and *trans*-processing of gag-precursor $Pr53^{gag}$ substrate in *in vitro* assays (*see* Fig. 4, Table 1, Pichuantes *et al.*, Proteins. 6:324-37 (1989))

(iii) From a review of the specification, one of skill in the art would understand that fusion of heterologous hSOD polypeptide sequence to a truncated NS2/3 protein produced a hybrid protein that was proteolytically cleaved.

37. It has been observed that fusion of a heterologous polypeptide sequence to a truncated fragment of a protein that by itself is inactive, can restore activity of the protein fragment. A fragment containing the first domain of the CD45 protein lacks phosphatase activity, but fusion of this fragment to maltose-binding protein restores the phosphatase activity. Lorenzo *et al.*, FEBS. 411(2-3):231-5 (1997).

38. Fusion with hSOD had been observed to stabilize the HIV protease. (*see* Pichuantes *et al.* J. Biol. Chem 265(23), at p.13892, col. 2 (1990))

39. The fusion of the NS2/3 fragments containing 299, 513 or 686 residues downstream from residue 946 to the 151 amino acids long hSOD fragment led to self-cleavage within the NS2/3 sequence.

40. One of skill in the art would understand from Example 5 in the specification, that fusion of the heterologous hSOD sequence to the NS2/3 fragments containing the 299, 513 or 686 residues allowed proteolytic cleavage within the NS2/3 sequence.

41. The crystal structure of the NS2/3 protease indicates that it forms a critical dimer interface comprising the residues His-952 and Glu-972 located in the loop region following helix H2 in the amino-terminal subdomain, and Cys-993 located in the b1–b2 loop of the C-terminal subdomain. (Lorenz et al., Nature 442:831-835 (2006), at para 3 of left column and para 1 of right column and Figures 1 and 2). The NS3 domain sequence of Figure 1 on the '691 application includes these amino acids involved in forming the active site of the NS2/3 protease.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Executed this 7 th day of February, 2007.

J.-H. James Ou, Ph.D.

CURRICULUM VITAE

JING-HSIUNG JAMES OU, Ph.D.

PERSONAL INFORMATION

Date of Birth:	June 10, 1954
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EDUCATION

Ph.D., 1982, Division of Biology, California Institute of Technology.

B.S., 1976, Department of Zoology, National Taiwan University.

PROFESSIONAL APPOINTMENTS

Professor, Department of Molecular Microbiology and Immunology, University of Southern California, 1999 to present.

Associate Director, Hepatitis C Cooperative Research Center, University of Southern California, 1996 to 2005.

Visiting Professor, Institute of Molecular Biology, Academia Sinica, Taiwan, May-August 2001

Associate Professor, Department of Molecular Microbiology and Immunology, University of Southern California, 1992 to 1999.

Assistant Professor, Department of Microbiology, University of Southern California, 1986-1992.

Postdoctoral Fellow, Department of Biochemistry and Biophysics, University of California, San Francisco, 1982-1986.

SCIENTIFIC ADVISORY BOARD

Member, Editorial Advisory Board, *Journal of Infectious Diseases*, 2006-present

Member, Editorial Board, *Journal of Virology*, 2004-2009

Member, Advisory Board, *Archives of Virology*, 2004-2006

Member, Editorial Board, *Hepatology*, 2004-2006

Member, Editorial Board, *Virology*, 2001-2006

Member, Editorial Advisory Board, *Infection Control Today*, 2002

Member, Special Emphasis Panel (ZDA1 RXL-E (03)), National Institutes of Health, October 24, 2006

Member, Special Emphasis Panel (ZDA1 RXL-E (02)), National Institutes of Health, October 23, 2006

Member, Scientific Review Committee, National Health Research Institutes, Taiwan, August 21-25, 2006

Member, Scientific Review Committee for National Research Program for Genomic Medicine, National Science Council, Taiwan, Dec. 7-9, 2005

Member, Scientific Review Committee, National Health Research Institutes, Taiwan, August 22-25, 2005

Reviewer, Singapore Biomedical Research Council (A*STAR) Grant Application, 2005, 2006

Member, Special Emphasis Panel (ZRG1 IDM-G(90)), National Institutes of Health, June 2-3, 2005

Member, National Cancer Institute Site-visit team (NCI-A RTRB-R (Y3)), February 22-24, 2005

Member, Scientific Review Committee for National Research Program for Genomic Medicine, National Science Council, Taiwan, Jan. 5-6, 2005

Member, Special Emphasis Panel (ZRG1 IDM-G(90)), National Institutes of Health, June 3-4, 2004

Member, Scientific Review Committee for Genomics, National Science Council, Taiwan, December 5-6, 2003

Reviewer, Scientific Research Program, Swiss National Science Foundation, 2003

Member, Scientific Review Committee, National Health Research Institutes, Taiwan, August 12-14, 2003

Member, Experimental Virology Study Section (ZRG1 EVR 90), National Institutes of Health, July 7-8, 2003

Reviewer, Scientific Program in Infection and Immunity, Wellcome Trust, London, United Kingdom, 1999, 2001, 2002, 2003, 2004, 2005

Member, Review Panel for Genomics, National Science Council, Taiwan, December 13-14, 2002

Member, Site-Visit Team (ZCA1 GRB-I (J1)), National Cancer Institute, November 2002

Member, Scientific Review Committee, National Health Research Institutes, Taiwan, September 4-7, 2002

Member, Site-Visit Team, National Health Research Institutes, Taiwan, September 2-3, 2002

Member, National Institute of Drug Abuse Special Emphasis Panel (ZDA1-RXL-E-07), August 2002

External Reviewer, McLaughlin Fellowship Fund, University of Texas Medical Branch, Galveston, Texas, May 2002, 2003, 2004, 2005

Member, Site-Visit Team (ZDA1 RXL-E (94)), National Institute of Drug Abuse, April 2002

Ad hoc reviewer, National Research Program for Genomic Medicine, National Science Council, Taiwan, March 2002

Reviewer, Research Grants Council of Hong Kong, Hong Kong, February 2002, 2003, 2004, 2005, 2006

Ad hoc reviewer, the Merit Review Subcommittee for Infectious Diseases, the Department of Veterans affairs, 1999, 2000, 2001

Member, National Institutes of Health Special Emphasis Panel (ZDA1-KXN-G-16), July 2000

Reviewer, The Cancer Research Campaign, London, United Kingdom, July 2000

Member, National Cancer Institute Site-Visit Team (NCI-A GRB-H (T4)), March 2000

Ad hoc member, National Institutes of Health, Experimental Virology Study Section, October 1999.

Ad hoc reviewer, Frontier Science, National Science Council, Taiwan, June 1999.

Member, National Institutes of Health, Experimental Virology Special Study Section for Postdoctoral (F32) Fellowships, October 1998, February and July 1999.

Member, the Merit Review Subcommittee for Infectious Diseases, the Department of Veterans Affairs, U.S.A., 1995-98.

Member, the Scientific Advisory Board, Center for Multiple Hepatitis Virus Infections, Chang Gung Memorial Hospital and Medical College, Taipei, Taiwan, 1995-98.

Member, National Institutes of Health Special Emphasis Panel (ZAI1-PRJ-M-C2), October 1998.

Member, National Cancer Institute Site-Visit Team, May 1997.

University of Southern California Committees

Member, University Committee on Appointment, Promotion and Tenure (UCAPT), 2001-2006

Director, M.S. Degree Program, Department of Molecular Microbiology and Immunology, 1995-2006

Member, Graduate Council, Keck School of Medicine, 1995-2006

Member, Executive Committee, Systems Biology and Diseases Interdisciplinary Graduate Program, Keck School of Medicine, 2004-2006

Member, M.D./Ph.D. Student Committee, Keck School of Medicine, 1991-2003

Member, Basic Science Seminar Committee, 1987-2000

Member, Scientific Council, Keck School of Medicine, 1993-1998

Member, Norris Cancer Center Scientific Council, 1991-1996

HONORS AND AWARDS

Invited Speaker, Viral Oncogenes, Santa Fe, New Mexico, September 15-18, 2005

Keynote Speaker, Annual Meeting of the Southern California Chapter of Society for Chinese Bioscientists in America, University of California, Irvine, October 2002

Keynote Speaker, Twentieth Anniversary of Chang Gung Memorial Hospital, Taiwan, 1996

Certificate in Recognition of Excellence in Teaching, The Class of 1997 and the Associated Students, USC School of Medicine, 1995

Robert E. and May R. Wright Foundation Research Award, 1995

Cathay Hepatitis Research Achievement Award, Society of Chinese Bioscientists in America, Singapore, 1992

Robert E. and May R. Wright Foundation Research Award, 1989

USC Faculty Research And Innovation Award, 1987

Robert E. and May R. Wright Foundation Research Award, 1987

Li Ming Graduate Research Achievement Award, California Institute of Technology, 1979

Arthur MaCallum Graduate Fellowship, California Institute of Technology, 1977-1982

PROFESSIONAL SOCIETIES

American Society for Microbiology, 1987-present

American Society for Virology, Life Member

American Society for the Advancement of Science, 1987-present

Society of Chinese Bioscientists in America, Life Member

Councilor, Society of Chinese Bioscientists in America, January 2004 – December 2007

PUBLICATIONS

1. Ou, J.H., Strauss, E.G. and Strauss, J.H. (1981) Comparative studies of the 3'-terminal sequences of several alphavirus RNAs. *Virol.* 109:281-289.

2. Ou, J.H., Rice, C.M., Dalgarno, L., Strauss, E.G. and Strauss, J.H. (1982) Sequence studies of several alphavirus genomic RNAs in the region containing the start of the subgenomic RNA. *Proc. Natl. Acad. Sci. USA* 79:5235-5239.

3. Monroe, S.S., Ou, J.H., Rice, C.M., Schlesinger, S., Strauss, E.G. and Strauss, J.H. (1982) Sequence analysis of cDNA's derived from the RNA of Sindbis virions and of defective interfering particles. *J. Virol.* 41: 153-162

4. Ou, J.H., Trent, D.W. and Strauss, J.H. (1983) The 3'-noncoding regions of alphavirus RNAs contain repeating sequences. *J. Mol. Biol.* 156: 719-730.

5. Ou, J.H., Strauss, E.G. and Strauss, J.H. (1983) The 5'-terminal sequences of the genomic RNAs of several alphaviruses. *J. Mol. Biol.* 168: 1-15.

6. Rutter, W.J., Ziemer, M., Ou, J., Shaul, Y., Laub, O., Garcia, P.D. and Standring, D.N. (1984) Transcription units of hepatitis B virus genes and expression of integrated viral sequences. In "Viral hepatitis and liver diseases", eds. G.N. Vyas, J.L. Dienstag, J.H. Hoofnagle, pp. 67-86 (Grune & Stratton, Inc.)

7. Ou, J.H. and Rutter, W.J. (1985) Hybrid hepatitis B virus-host transcripts in a human hepatoma cell. *Proc. Natl. Acad. Sci. USA* 82: 83-87.

8. Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Graf, L., Clauser, E., Ou, J.H., Marsiarz, F., Kan, Y.W., Goldfine, I.D., Roth, R.A. and Rutter, W.J. (1985) The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell* 40:747-758.

9. Ou, J.H., Laub, O. and Rutter, W.J. (1986) Hepatitis B virus gene functions: the precore region targets the core antigen to cellular membranes and causes the secretion of the e antigen. *Proc. Natl. Acad. Sci. USA* 83: 1578-1582.

10. Ou, J.H. and Rutter, W.J. (1986) The role of the precore region of the hepatitis B virus genome in the compartmentalization of core gene products. In "Viruses and human cancer", eds. R.C. Gallo, W. Haseltine, G. Klein, H. zur Hausen, pp. 479-491. (Alan R. Liss, Inc., New York)

11. Wang, K.S., Choo, Q.L., Weiner, A.J., Ou, J.H., Najarian, R.C., Thayer, R.M., Mullenbach, G.T., Denniston, K.J., Gerin, J.L. and Houghton, M. (1986) The structure, sequence and expression of hepatitis delta viral genome. *Nature* 323: 508-512.

12. Wang, K.-S., Choo, Q.L., Weiner, A.J., Ou, J.H., Denniston, K.J., Gerin, J.L. and Houghton, M. (1987) The viroid-like structure of the hepatitis delta genome: synthesis of a viral antigen in recombinant bacteria. *Progress in Clinical & Biological Research.* 234:71-82.

13. Standring, D.N., Ou, J.H. and Rutter, W.J. (1986) Assembly of viral particles in Xenopus oocytes: presurface antigens regulate secretion of the hepatitis B viral surface envelope particle. *Proc. Natl. Acad. Sci. USA* 83: 9338-9342.

14. Standring, D.N., Ou, J.H. and Rutter, W.J. (1987) Expression of hepatitis B viral antigens in Xenopus oocytes. In "Hepadna Viruses", eds. W. Robinson, K. Koike & H. Will, pp. 117-127. (Alan R. Liss, Inc., New York)

15. Ou, J.H. and Rutter, W.J. (1987) Regulation of secretion of the hepatitis B virus major surface antigen by the preS-1 protein. *J. Virol.* 61:782-786.

16. Ou, J.H., Yen, T.S.B., Wang, Y.F., Kam, W.K. and Rutter, W.J. (1987) Cloning and characterization of a human ribosomal protein gene with enhanced expression in fetal and neoplastic cells. *Nucl. Acid. Res.* 15:8919-8934.

17. Garcia, P.D., Ou, J.H., Rutter, W.J. and Walter, P. (1988) Targeting of the hepatitis B virus precore protein to the endoplasmic reticulum membrane: after signal peptide cleavage translocation can be aborted and the product released into the cytoplasm. *J. Cell Biol.* 106:1093-1104.

18. Ou, J.H.*, Standring, D.N.*, Masiarz, F. and Rutter, W.J. (1988) A signal peptide encoded within the precore region of hepatitis B virus directs the secretion of a heterogeneous population of e antigens in Xenopus oocytes. *Proc. Natl. Acad. Sci. USA* 85:8405-8409. (*Co-first authorship)

19. Seto, E., Yen, T.S.B., Peterlin, B.M. and Ou, J.H. (1988) Trans-activation of the human immunodeficiency virus long terminal repeat by the hepatitis B virus X protein. *Proc. Natl. Acad. Sci. USA* 85: 8286-8290.

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